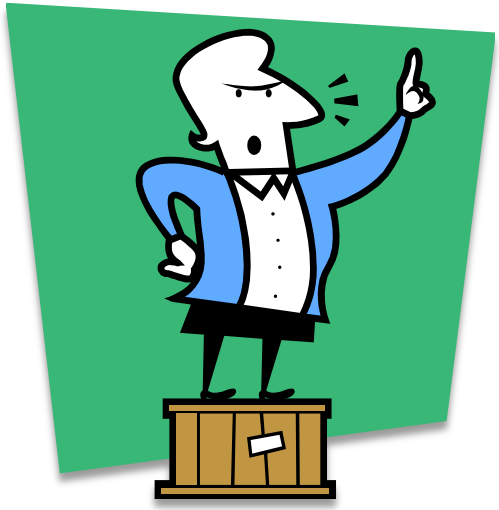
Thema der Vorlesung

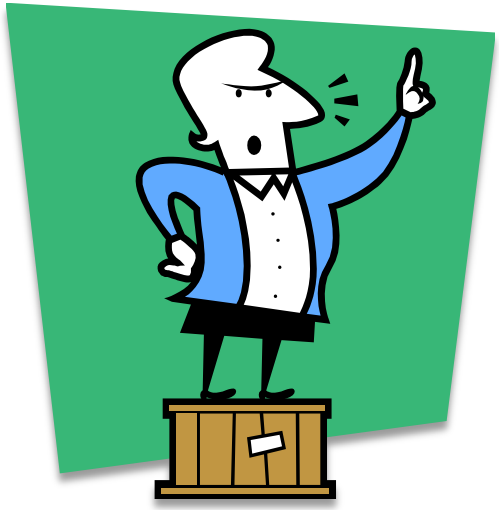


Sekretion von Proteinen

Bakterielle Genomik

Thema der Vorlesung

Sekretion von Proteinen

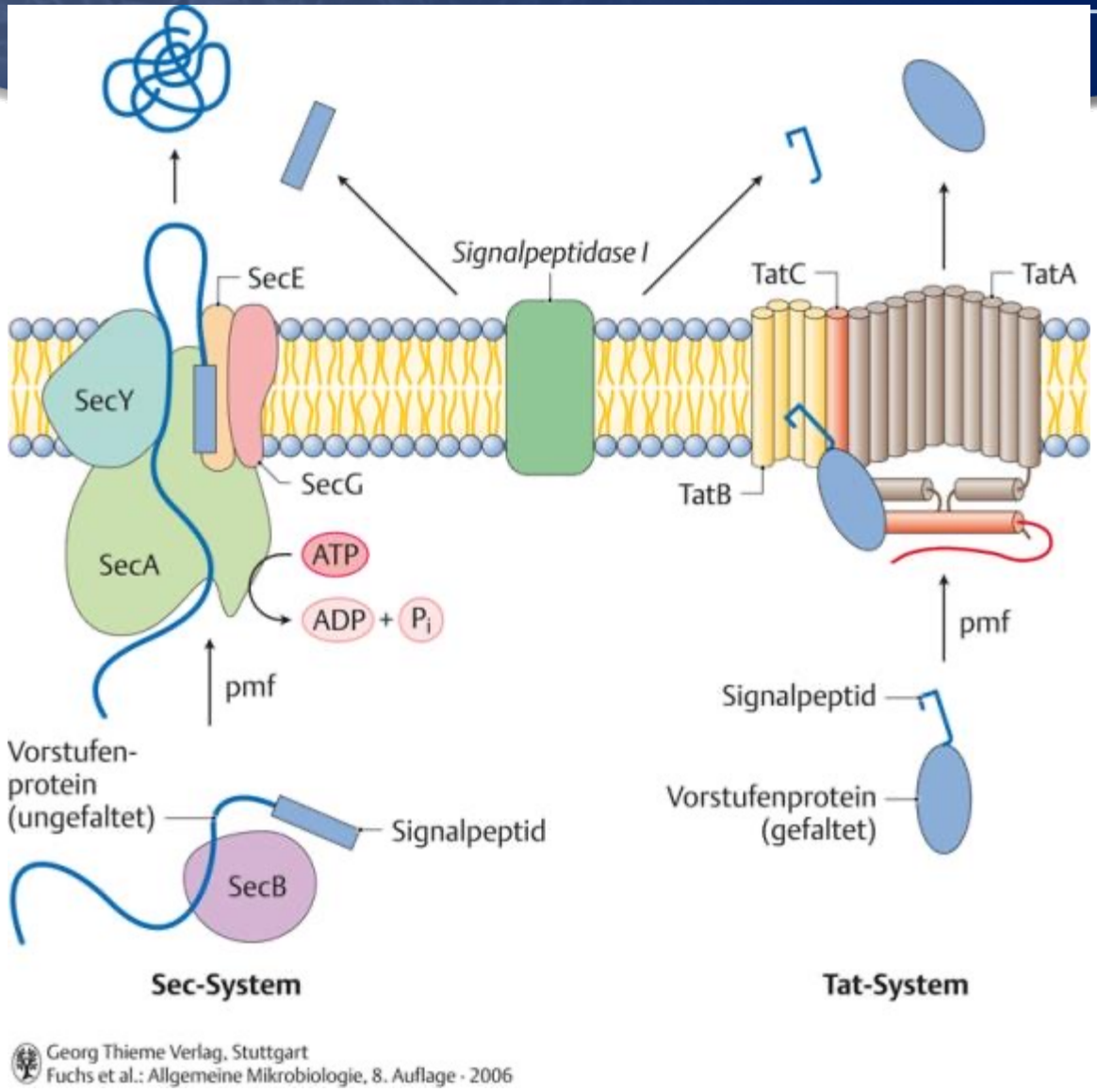


Problem: Bis zu 50 % der Polypetide liegen außerhalb einer Zelle und müssen dorthin gelangen!

Daher besitzt die Zelle eine Reihe von Systemen, um Proteine gezielt an unterschiedliche Positionen außerhalb der Zelle zu bringen:
-in das Periplasma
-In die äußere Membran
-oder darüber hinaus in einen Wirt

Es gibt 3 Systeme für Transport über die innere Membran:

1. Sec, Transport und Faltung parallel
2. TAT (TwinArginin**M**otif) Tat verantwortlich für Transport von gefalteten, komplexen Proteinen über CM
3. SRP (Signal recognition pathway) SRP adhoc Translation und Secertion in die Membran-Sec nicht gefaltete Proteine



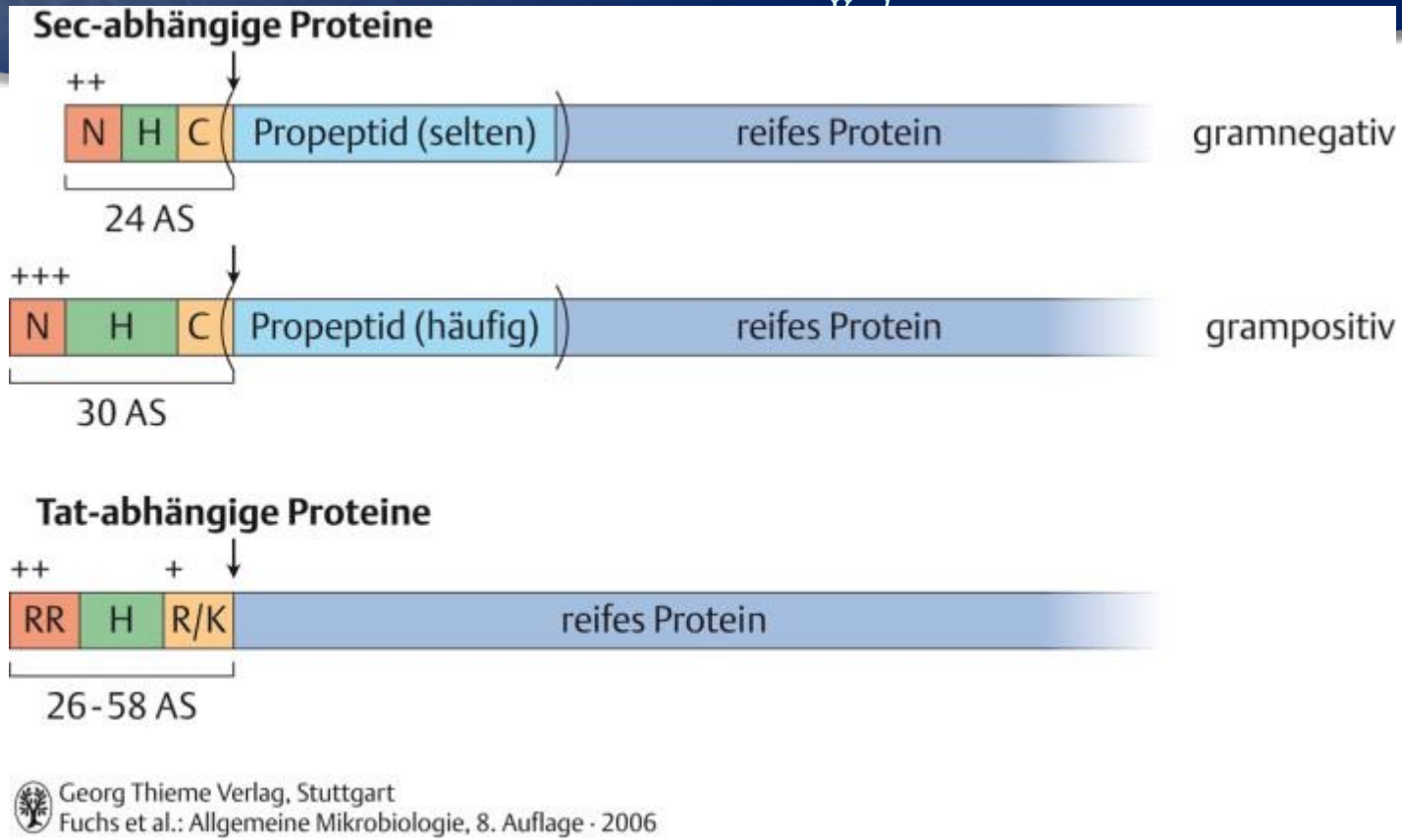
Georg Thieme Verlag, Stuttgart
Fuchs et al.: Allgemeine Mikrobiologie, 8. Auflage · 2006

SecA ist das wichtigste Protein; es führt das Protein and die Membran zum Transporter

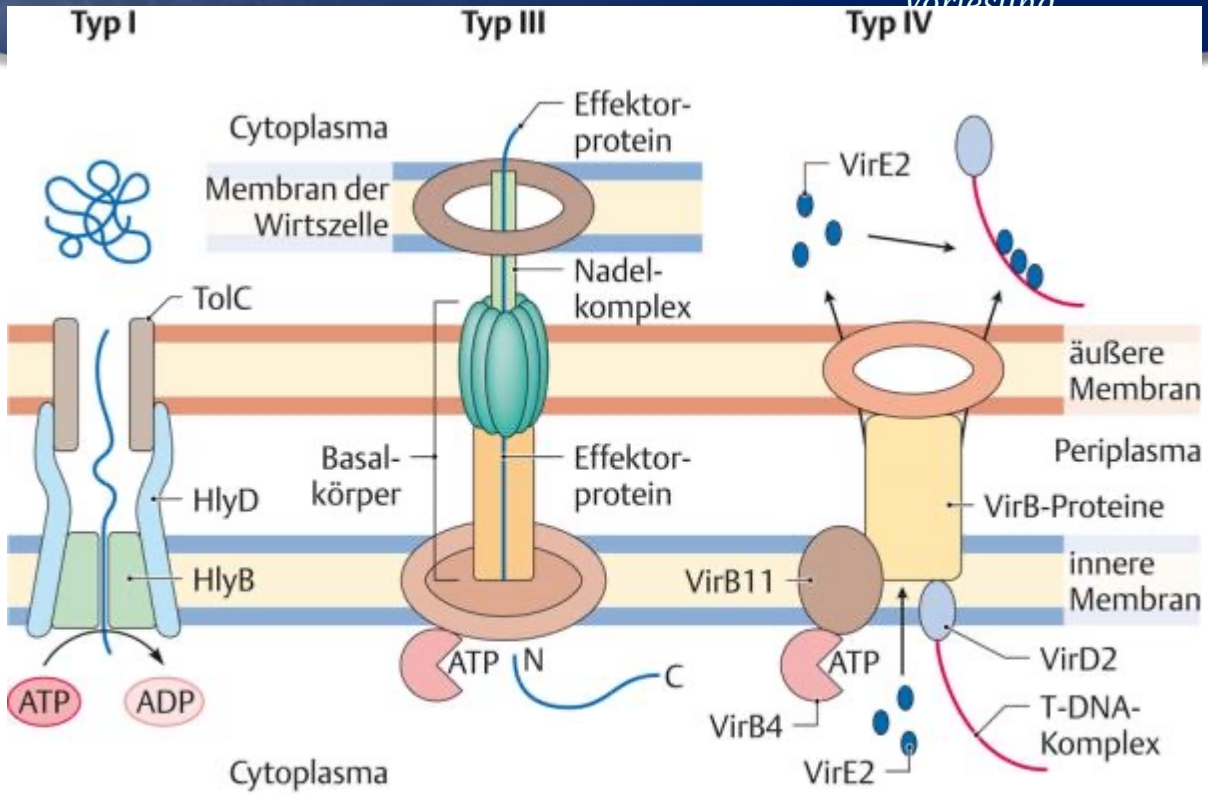
SecB ein Chaperon
SecE ist eine Protease
SecY und G - Hilfsproteine

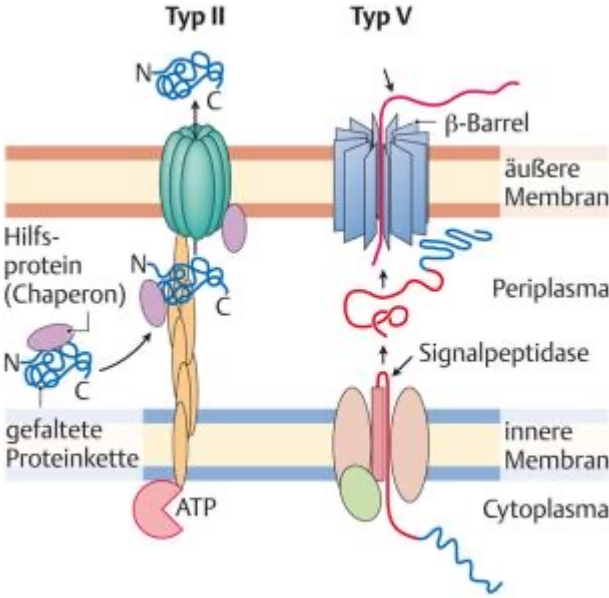
Energie durch PMF

TAT aus 3 Hauptproteinen:
Nur vollständig gefaltete Proteine (ca. 2-3 % aller sekretierten Proteine via TAT)



Erkennungssequenzen bestehen aus drei Domänen unterschiedlicher Ladung und Hydrophobizität N-terminale Domäne, H- Hydrophobe Domäne, C-terminale Domäne

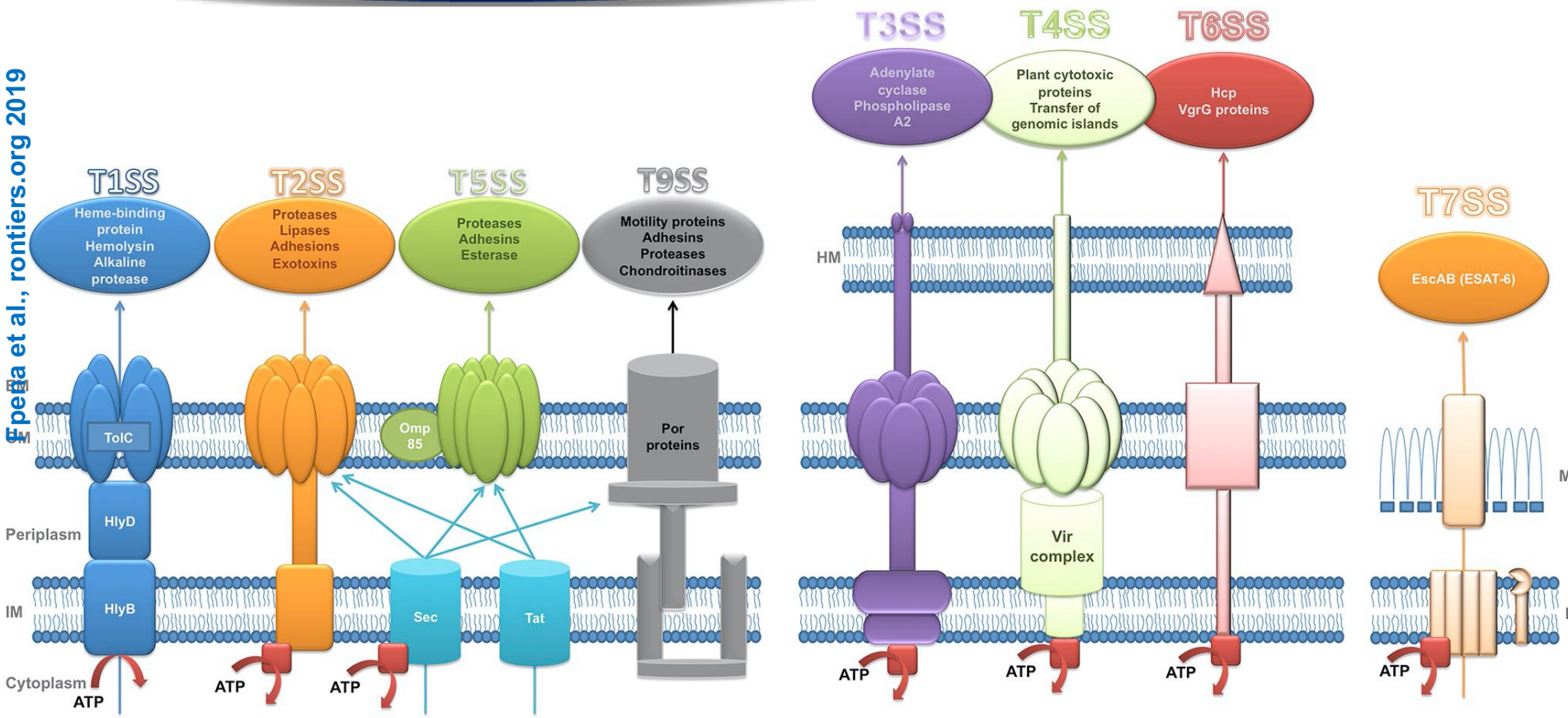




9 Transportsysteme für Effektormoleküle

I, III, IV mit Nadelartigen Strukturen

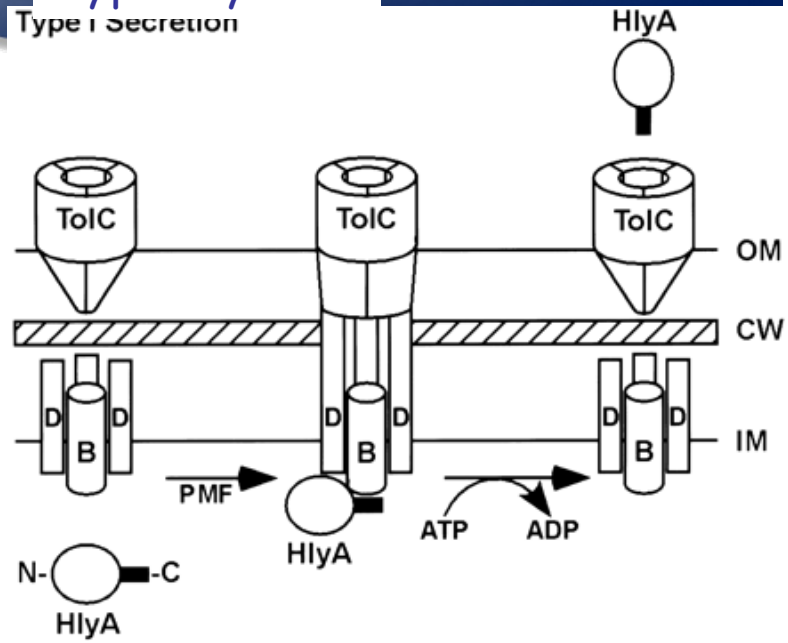
- Typ II und IV/ VI sind sec-abhängig

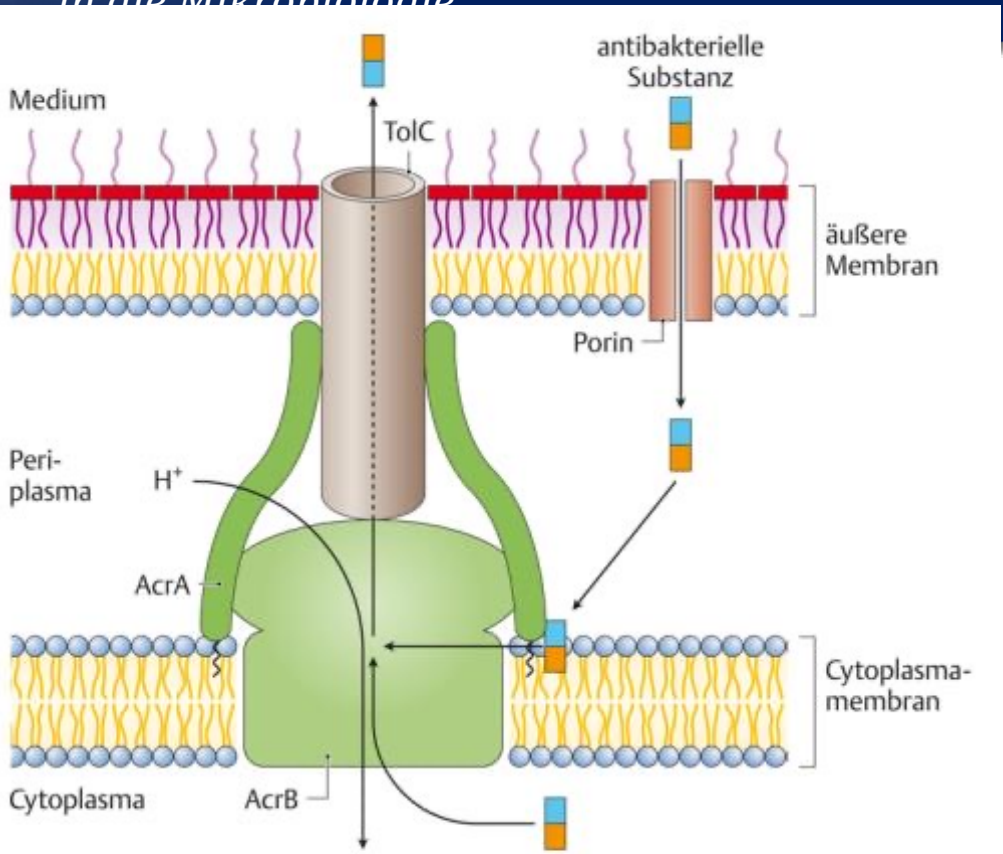


Epeña et al., rontiers.org 2019

Insgesamt 9 Transportsysteme für Effektoren
- Typ I und IV, IX sind sec-abhängig

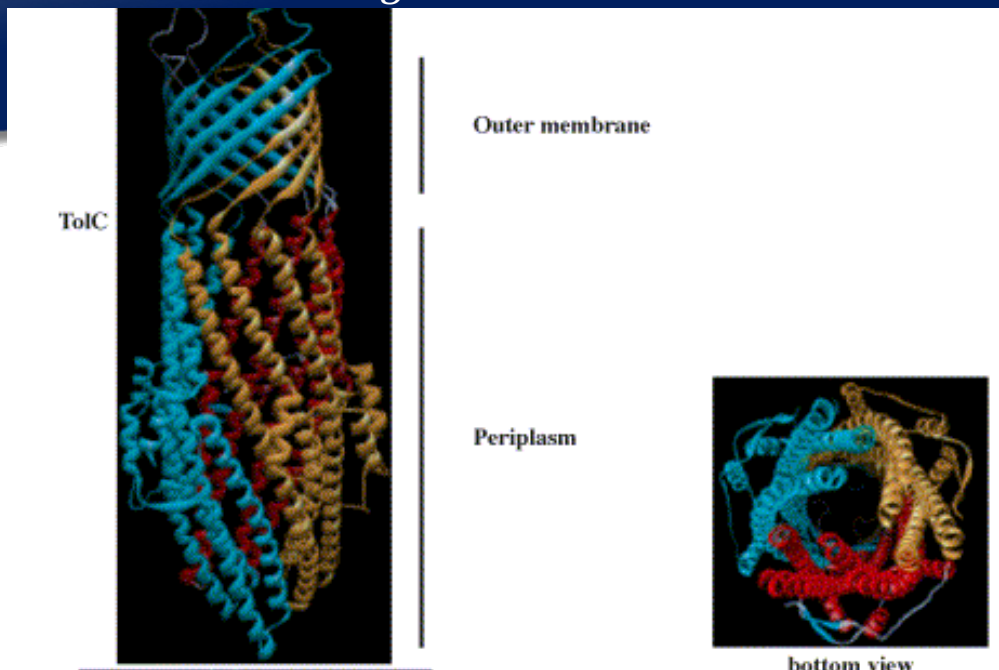
Typ I System





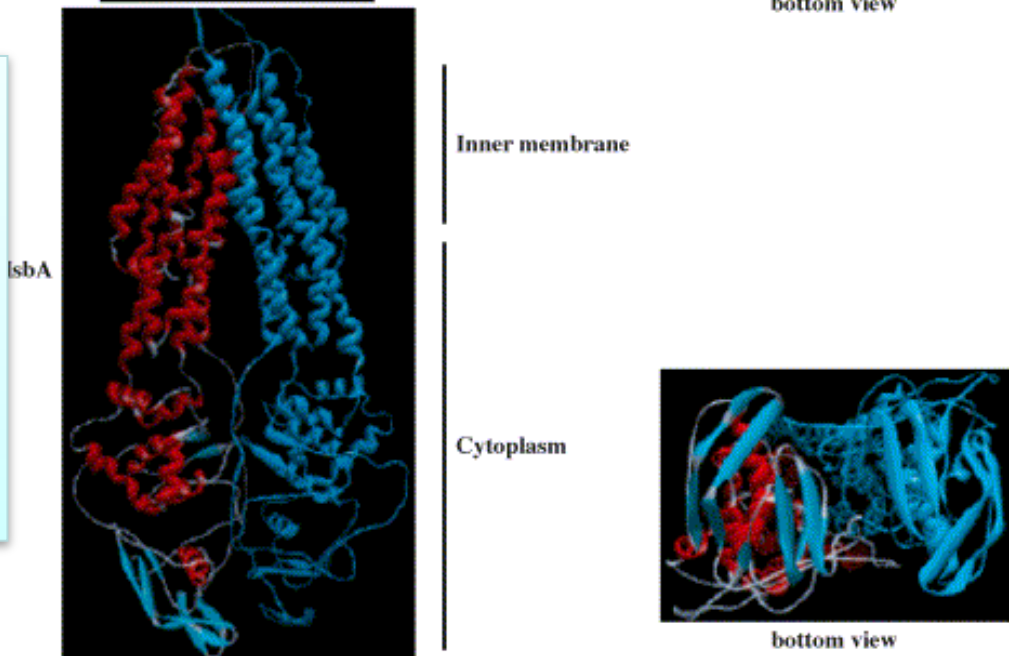
- HlyA (ArcAB) interagiert mit dem ABC Transporter bzw. der ATP-Bindecassette und HLYD, dem Membranbindeprotein (trimere Struktur)
- Translocation erfolgt simultan über CM und OM
- 11-17 AA Repeats in der Proteinsequenz für Anheftung wichtig; binden Ca2++
- Keine Prozessierung des Effektors
- Transport kontinuierlich; kein PM Intermediat des Effektors

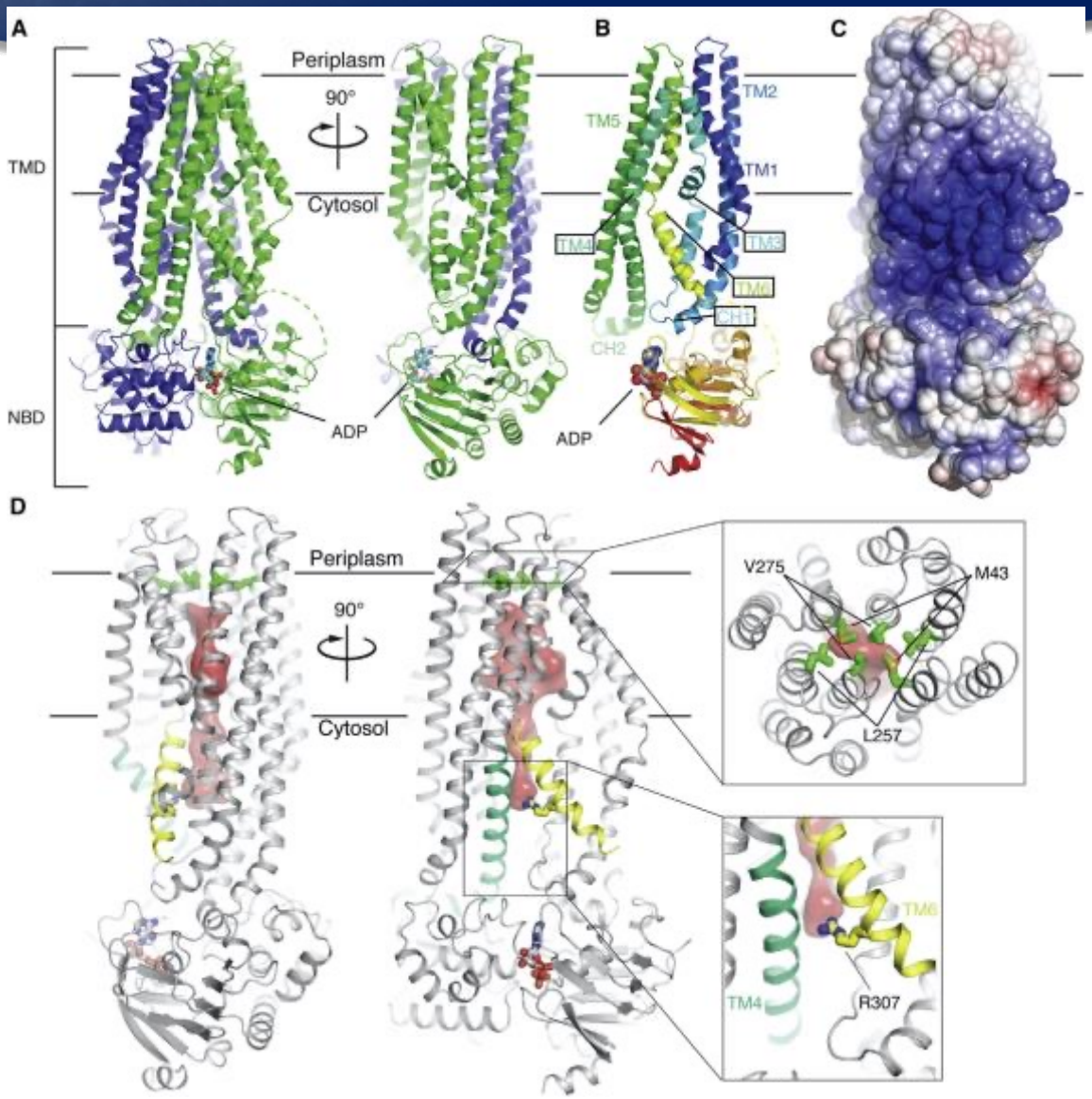
Beta sheets von TolC und MsbA aus *E. coli und V. cholera.* Beide Proteine sind ca 140 Ä gross; Beide Proteine sind TypI Transporter und bilden eine Pore



Hemolysin A-Transporter

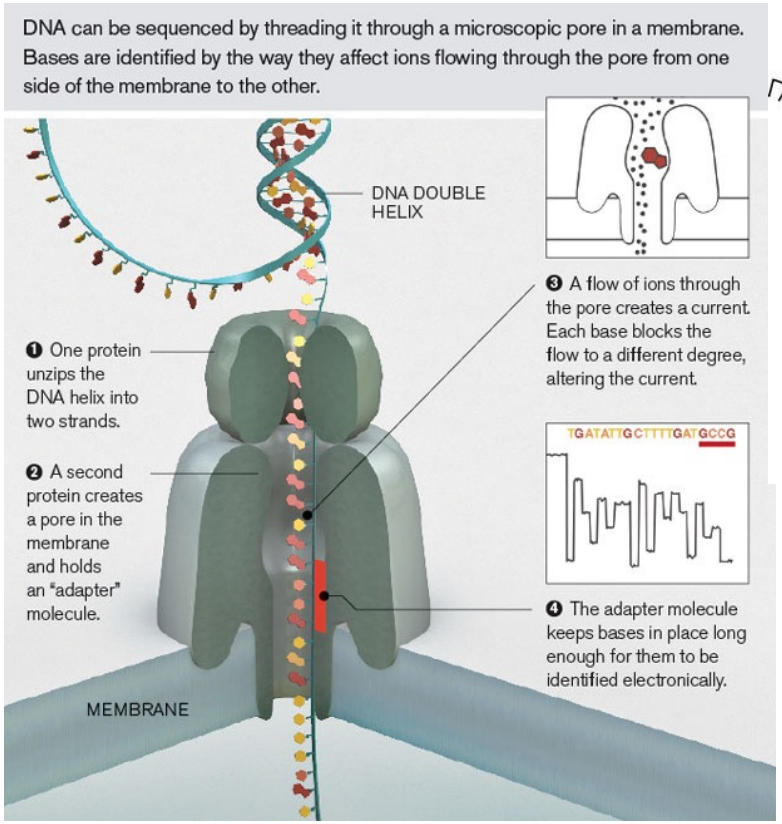
12 strand ß-barrel
140 Ä lang, ß-Barrel 40 Ä lang in der OM,
100 Ä – alpha-helikaler Bereich in CM und Periplasma
12 Helices

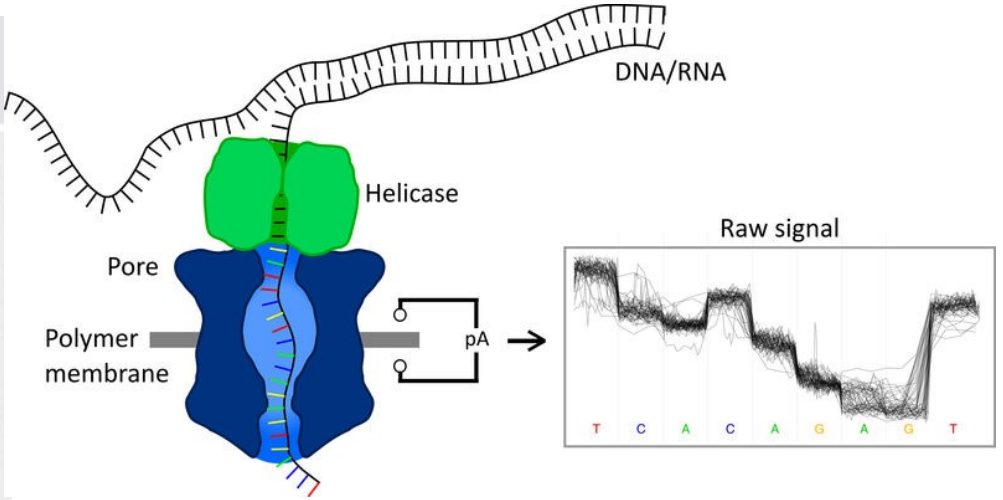




Überblick über die Struktur eines Typ I (ABC) Transporters aus *Aquifex aeolicus*

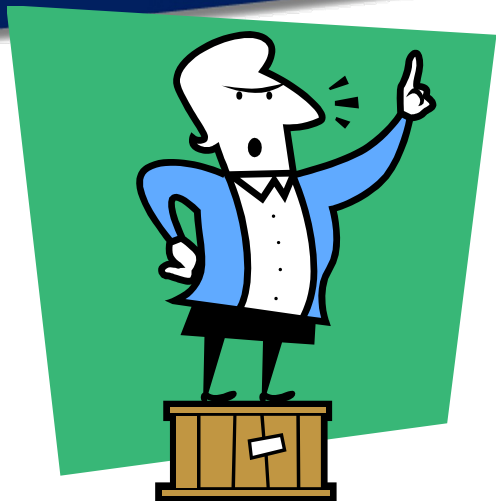
Morgan et al., Structure 25, 2017

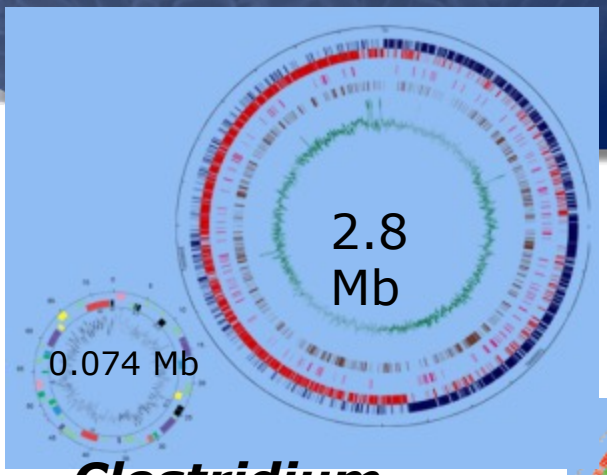

DNA can be sequenced by threading it through a microscopic pore in a membrane. Bases are identified by the way they affect ions flowing through the pore from one side of the membrane to the other.
DNA DOUBLE HELIX
❶ One protein unzips the DNA helix into two strands.
❷ A second protein creates a pore in the membrane and holds an "adapter" molecule.
❸ A flow of ions through the pore creates a current. Each base blocks the flow to a different degree, altering the current.
TGATATTGCTTTTGATGCCG
❹ The adapter molecule keeps bases in place long enough for them to be identified electronically.
MEMBRANE



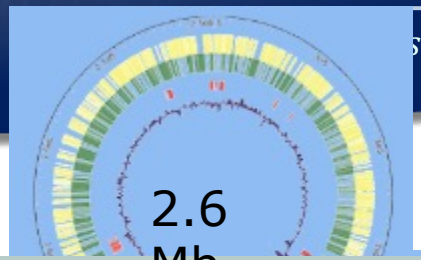
Oxford Nanopore Sequenzierung basiert auf demT1SS

Bakterielle Genomik

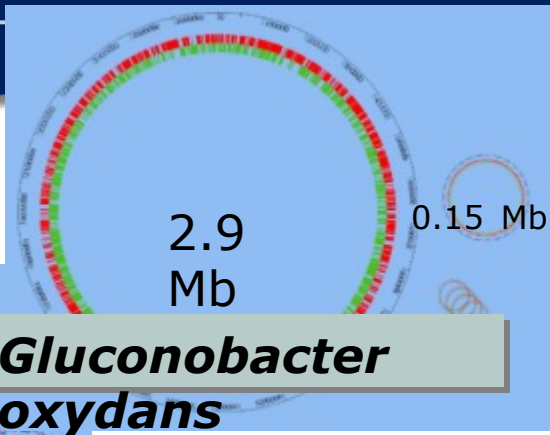




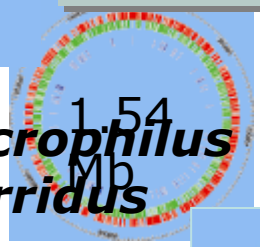
Clostridium tetani



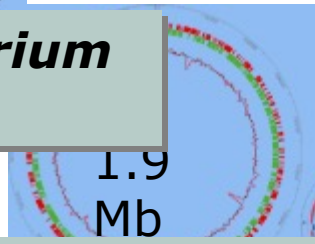
Propionibacterium acnes



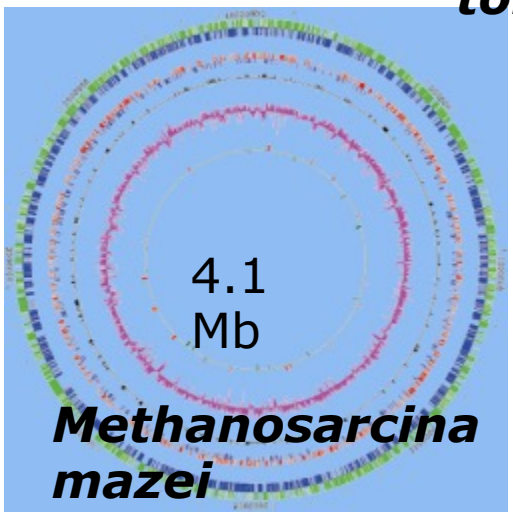
Gluconobacter oxydans



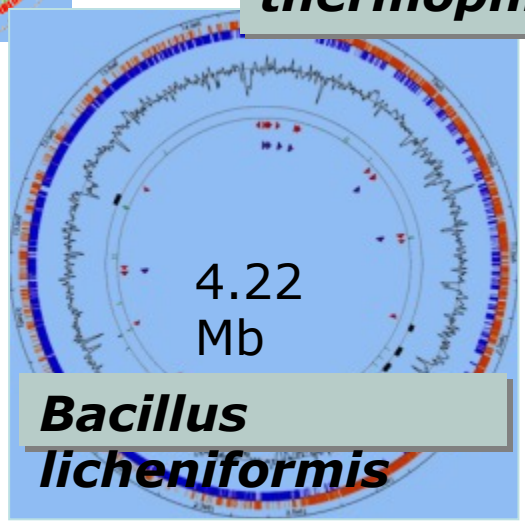
Picrophilus torridus



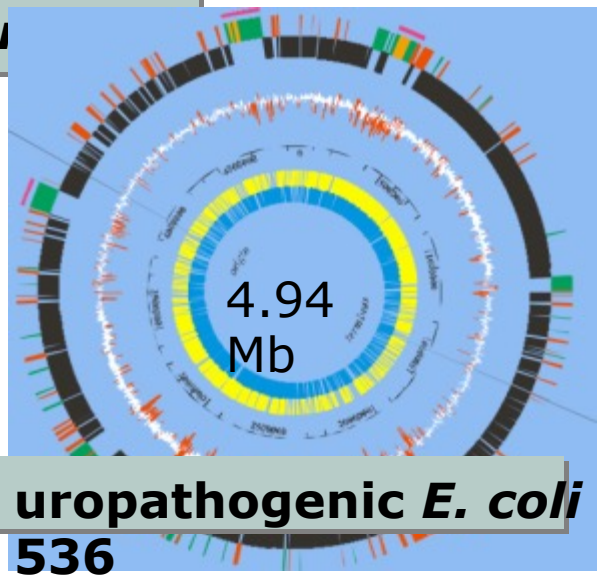
Thermus thermophilu



Methanosarcina mazei

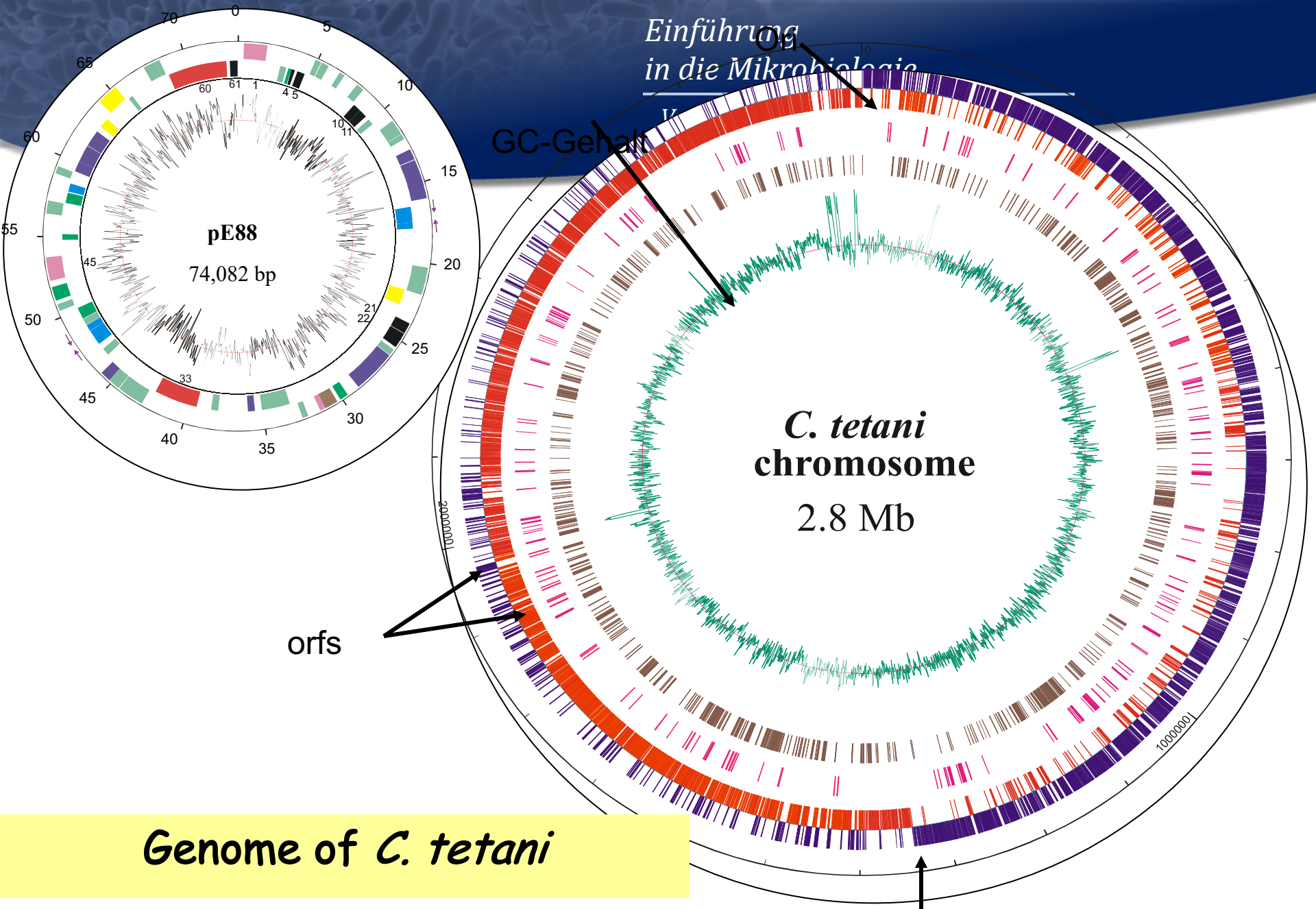


Bacillus licheniformis

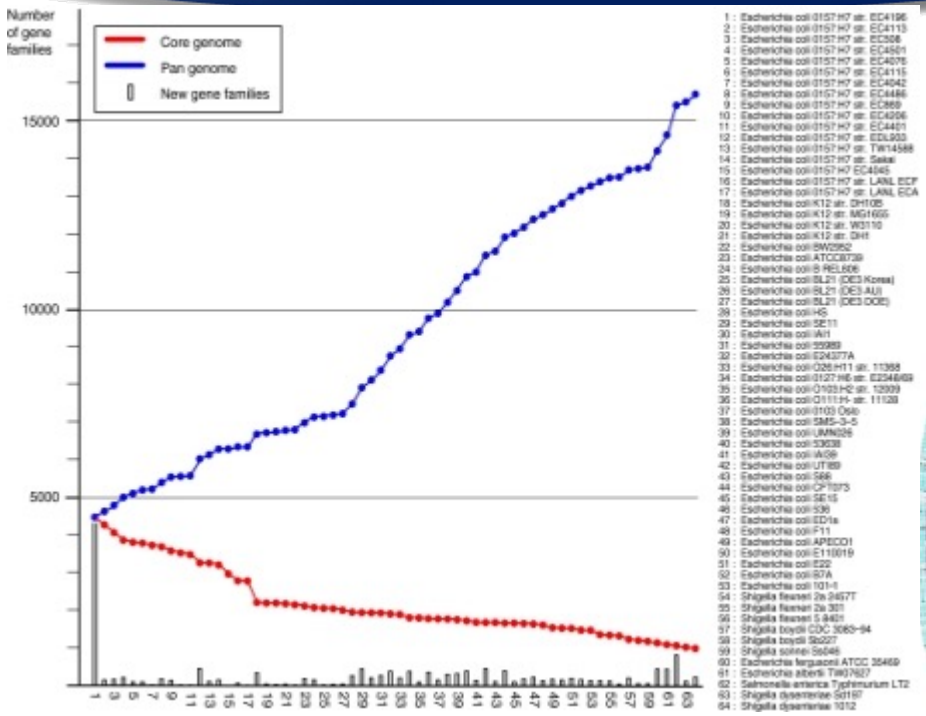


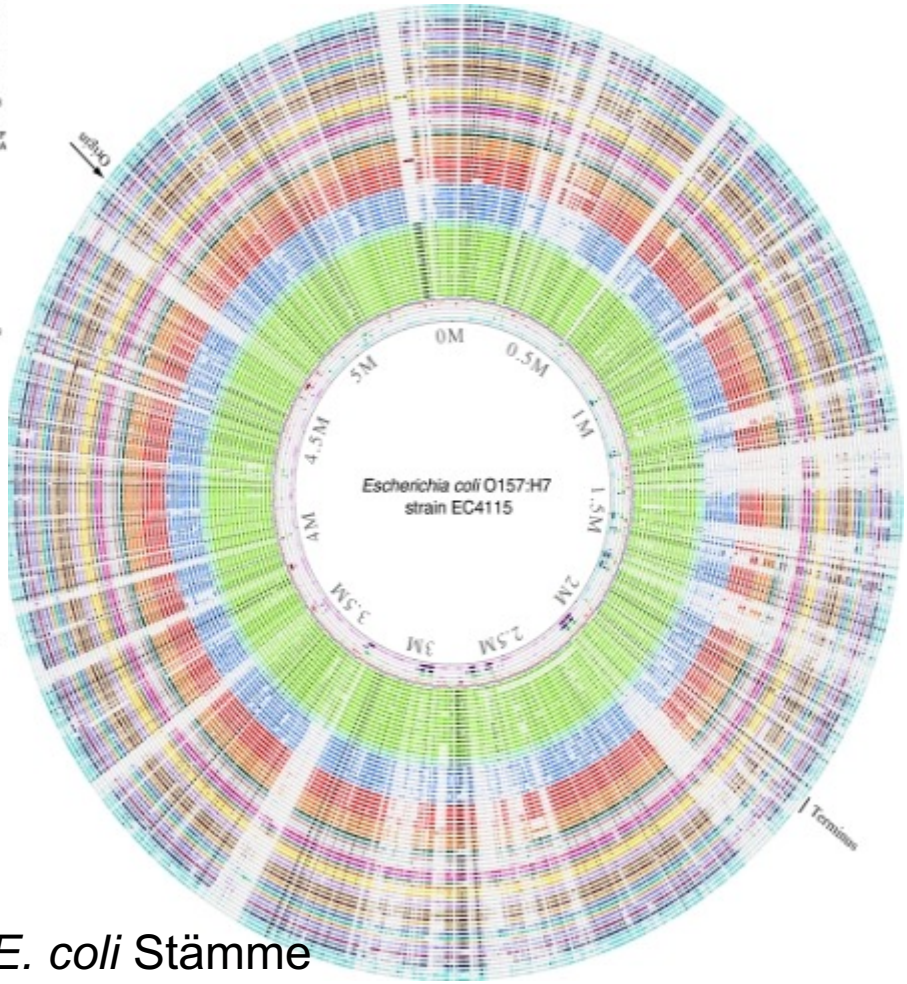
uropathogenic *E. coli* 536

Bakterielle Genome meist zirkulär bestehend aus Chromosomen und Megaplasmiden; nur wenige Beispiele für lineare Plasmide



Genome of *C. tetani*

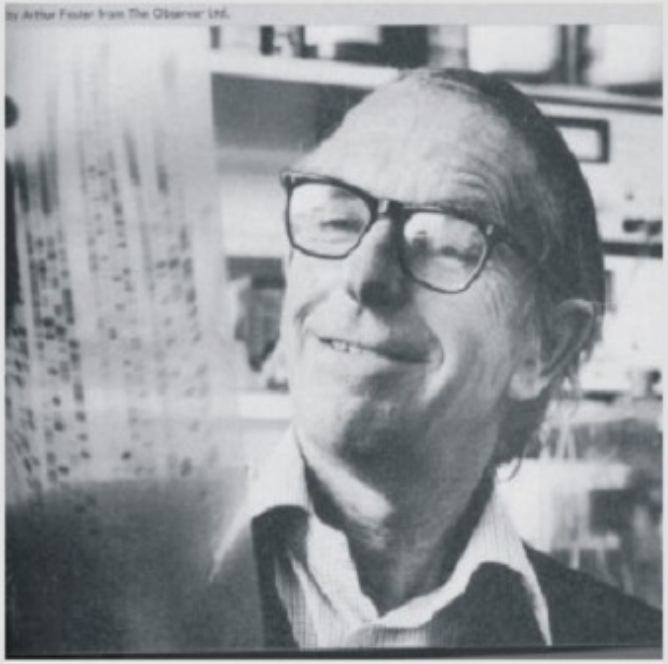




E. coli Pan- und Core Genom 61. versch. *E. coli* Stämme

Microb Ecol. 2010 Nov;60(4):708-20. Epub 2010 Jul 11.

DNA-Sequenzierung mit der Sanger-Technik



Frederick Sanger, 1977

Arten der Sequenzierung

- **„Kettenabbruch“- oder „Sanger“-Methode**
  Enzymatische Synthese markierter DNA, d.h. man beendet mit Hilfe bestimmter modifizierter Nukleotide, sogenannter Didesoxynukleotide, die Verlängerung des Stranges

- **Methode des „Chemischen Abbaus“ oder „Maxam-Gilbert“-Methode**
  Basenspezifischer chemischer Abbau eines DNA-Moleküls, das an einem Ende markiert ist

Heute:

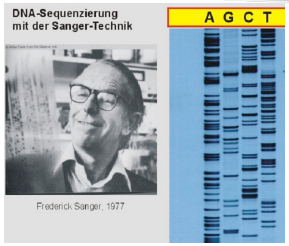
Pyrosequencing 454 Methode von LaR

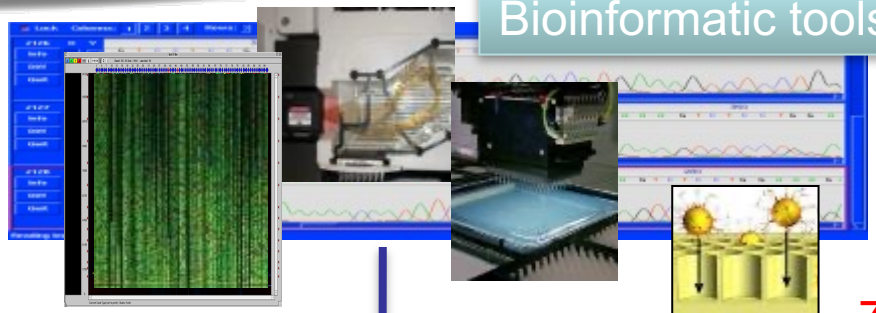
Illumina

und PacBio

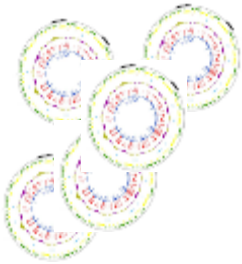
Oxford Nanopore

https://vimeo.com/127689865





Bioinformatic tools



70,000
genomes finnish

1973/74 1977 1983 1987 1995 2000
2018

F. Sanger

Modified dNTPs

PCR

Haemophilus influenzae
Fleischmann, Science

Fluororescent
dyes for all dNTPs

Capillary
Phi29 Polymerase
Robotics

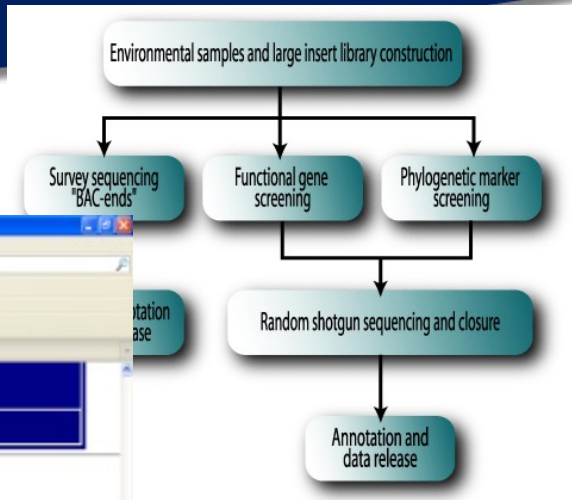
Solexa
Titanium
Ilumina…
Single Cell….

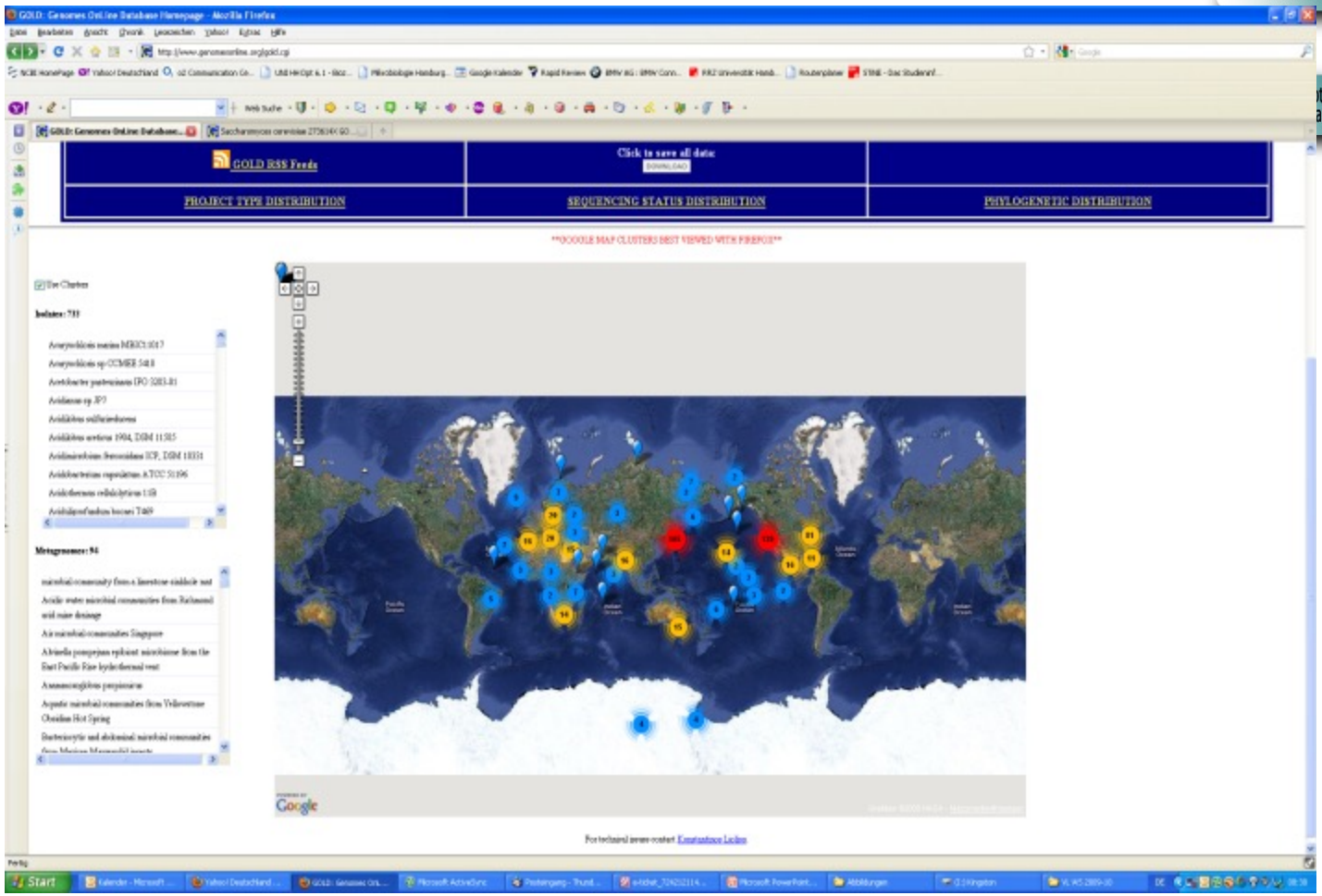
https://gold.jgi-psf.org

Verschiedene Genomgrößen: bp

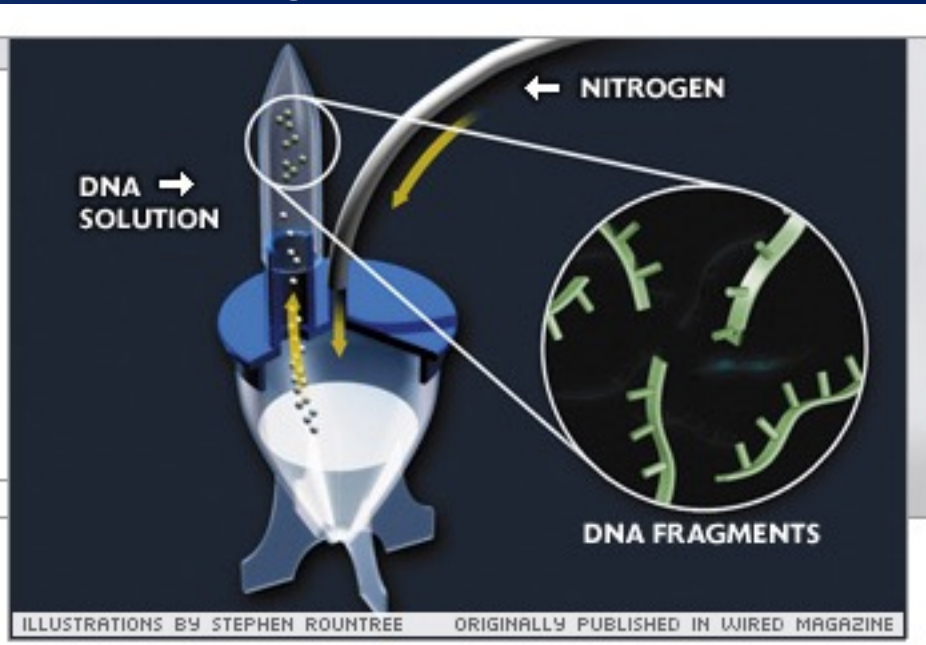
Haemophilus influenzae Rd	1.830.000
Mycoplasma genitalium G-37	580.000
Escherichia coli K12 MG1655	4.640.000
Archaeoglobus fulgidus DSM4304	2.180.000
Pseudomonas aeruginosa PA01	6.260.000
Escherichia coli O157:H7 Sakai	5.500.000
Methanosarcina mazei GÖ1	4.100.000
Clostridium tetani	2.800.000
Saccharomyces cerevisiae	11.600.00

Nächste Stufe der Genomsequenzierung:
Sequenzierung von mikrobiellen Habitaten.


Environmental samples and large insert library construction
Survey sequencing "BAC-ends"
Functional gene screening
Phylogenetic marker screening
Random shotgun sequencing and closure
Annotation and data release

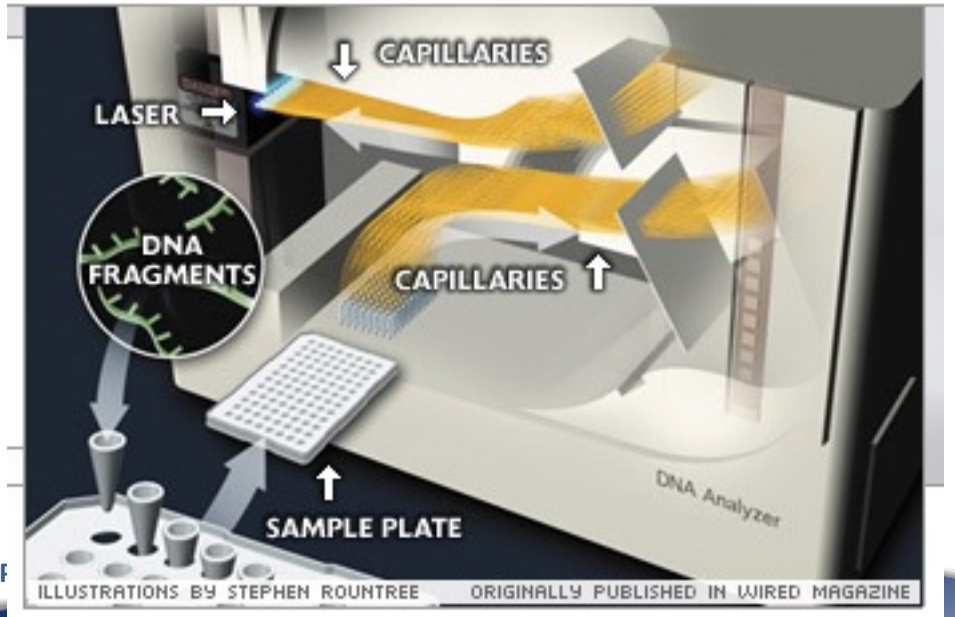


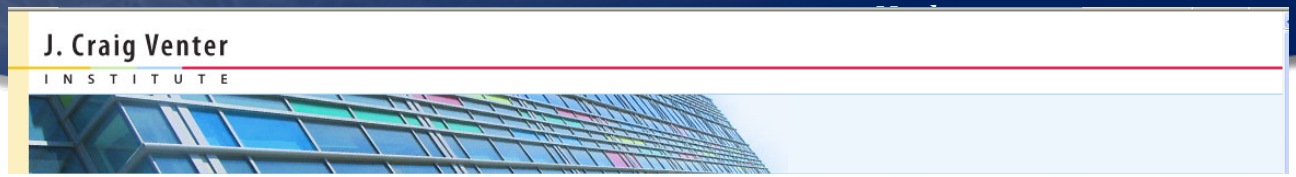
http://www.genomesonline.org/gold.cgi



Cosmid-Bibliotheken

BAC-Bibliotheken

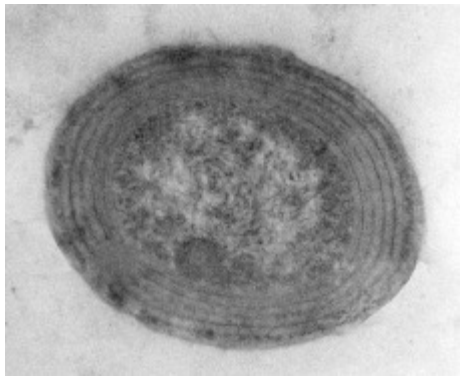


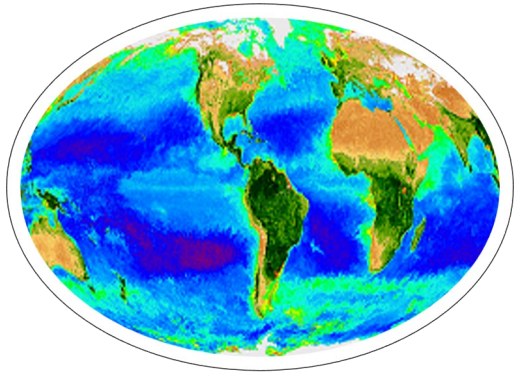


SORCERER II Expedition
EXPEDITION INFO
VOYAGE TRACKER
SAMPLING METHODS
SCIENTIFIC DATA
GLOSSARY
CURRENT LOCATION
Welcome to the Sorcerer II voyage tracking section.
Our tracking system gives you the ability to follow the progress of the expedition, read related articles and view photos from the voyage.
Please select a point from the map above to get started.
Note: Sampling points and route lines are approximations.
CURRENT LOCATION
ZOOM INTO A REGION
INFORMATION POINT
SELECTED POINT
SAMPLE LOCATION

Umweltgenom-Projekte Metagenomik

'The Most Important Microbe You've Never Heard' Science Magazine 2008





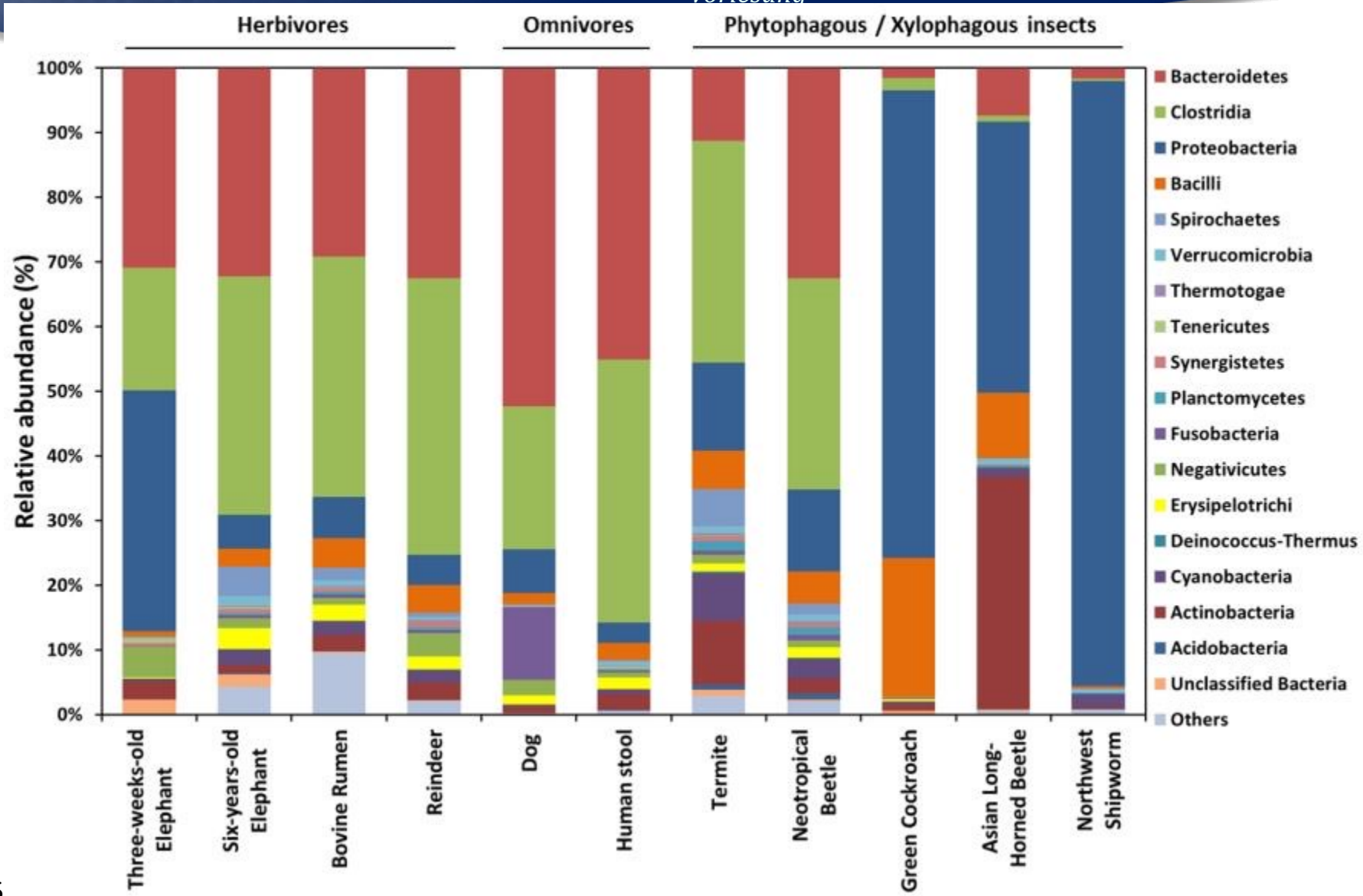
Prochlorococcus sp. just 0.5 to 0.8 micrometers across

100,000 cells per ml sea water; comes to around 10^{27} cells worldwide

20% of the oxygen produced in the atmosphere

Up to date 11 strains sequenced; grow in oligotrophic sea waters

G. Rocap, (2003). *Nature* **424**: 1042–1047.


Herbivores
Omnivores
Phytophagous / Xylophagous insects
Relative abundance (%)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Three-weeks-old Elephant
Six-years-old Elephant
Bovine Rumen
Reindeer
Dog
Human stool
Termite
Neotropical Beetle
Green Cockroach
Asian Long-Horned Beetle
Northwest Shipworm
Bacteroidetes
Clostridia
Proteobacteria
Bacilli
Spirochaetes
Verrucomicrobia
Thermotogae
Tenericutes
Synergistetes
Planctomycetes
Fusobacteria
Negativicutes
Erysipelotrichi
Deinococcus-Thermus
Cyanobacteria
Actinobacteria
Acidobacteria
Unclassified Bacteria
Others